Brief Report

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Tapeworm infection incidence in rural Japan points to a common environmental source of infection

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SUMMARY: *Dibothriocephalus nihonkaiensis* is a zoonotic tapeworm transmitted to humans through consumption of raw or undercooked fish or wild meat. Between 2022 and 2023, Yamagata Prefecture reported an increase in cases compared with 2017–2021, when none were observed. We conducted a clinical and environmental investigation to clarify infection sources. Four confirmed and one suspected patient were identified, all presenting with gastrointestinal symptoms. Exposures were linked to raw cherry salmon (*Oncorhynchus masou*) in three cases and undercooked bear meat in one case. Praziquantel treatment (10–20 mg/kg) was effective, with eight worms (76–210 cm) recovered. Environmental surveillance detected *D. nihonkaiensis* in 33.3% of bear feces (20/60) and 21.8% of wild fish samples (17/78). Phylogenetic analysis showed close genetic relatedness among human, bear, and fish isolates, indicating a shared transmission cycle. These findings confirm zoonotic transmission of *D. nihonkaiensis* in Yamagata and highlight the need for food safety awareness and environmental monitoring.

Keywords: Tapeworm infection, rural Japan, *Dibothriocephalus nihonkaiensis*, environmental source of infection

1. Introduction

Tapeworms, belonging to the class Cestoda, are complex parasitic flatworms with a life cycle involving intermediary hosts, frequently found in aquatic ecosystems (*1,2*). The consumption of raw or undercooked fish has long been recognized as a potential source of tapeworm infections, owing to the transmission of larval stages such as *Dibothriocephalus* spp. to humans (*1,2*). Japan's rich culinary heritage has long celebrated the art of sushi and sashimi, where the consumption of raw fish is a revered tradition (*1*). However, epidemiological data reveals a significant risk of tapeworm infections, and the primary source of *Dibothriocephalus* spp. infections in Japan are linked to the consumption of raw salmonids, particularly cherry salmon and immature chum salmon (*1,2*).

From the summer of 2022 to the spring of 2023, Yamagata Prefecture Central Hospital showed an increase in tapeworm cases compared to 2017-2021, when there were no reported cases. All cases are suspected to be associated with consuming raw or undercooked wild fish or bear meat. As part of an infection control strategy, an infection source tracking study was done, the results of which are described in this paper.

2. Methods

2.1. Environmental sample collection

Based on patients' epidemiological data, we conducted an environmental surveillance study in Yamagata Prefecture by collecting fecal samples from black bears living in wild forests and raw meat from wild fish in Yamagata rivers. In collaboration with hikers and recreational fishermen, a total of 60 bear fecal samples and 78 raw meat samples from wild fish were collected. Specimens were collected in 50-mL conical sampling tubes before immediate storage at −20°C. Samples were transferred to −80°C within one day of collection, where they were stored for up to 1 month.

2.2. Human sample collection

An adult worm of *D. nihonkaiensis* was isolated from an infected patient, and proglottids were recovered from the stool after treatment with praziquantel followed by a purge with $MgSO_4$ solution.

2.3. DNA extraction

A single proglottid was finely chopped and put in the tissue lysis buffer of the DNA extraction kit. Total genomic DNA was extracted using a DNeasy tissue Kit, Qiagen (Germany) according to the manufacturer's instructions. Protocols for bear fecal and wild fish sample processing and extraction were adapted from previously published procedures (*1,2*). Between 10-20 mg of sample (fecal sample/chopped proglottid) was added to a 2-mL screw cap tube with a rubber O-ring (Corning,USA). Next, 1,200 µL of PowerProtect DNA/RNA (Qiagen, Germany) and 0.5 g silica zirconia beads (BioSpec , Singapore) were added, and the samples were homogenized using a BioSpec bead beater (BioSpec, Singapore). Between 100-200 µL of supernatant was filtered through a Centricon® Plus-70 centrifugal ultrafilters (100 kDa cut off; Merck Millipore, USA) *via* centrifugation at 1,500×g for 15 minutes at 4°C, with a resulting concentrate ranging between 2.11- 4.13 g. To quantify DNA concentration, 1 mL of well-mixed Centricon® (Merck Millipore, USA) concentrates were added directly to a commercial kit optimized for isolation of total DNA from environmental samples according to the manufacturer's protocol (DNeasy tissue Kit, Qiagen, Germany) (*3*). Two replicate DNA extractions and analyses were performed for each sample. Isolated DNA pellets were dissolved in 50 µL of deoxyribonuclease-free water, and total DNA was measured by spectrophotometry (NanoDrop, Thermo Fisher Scientific) as previously described (*3*).

2.4. Molecular identification by real-time qPCR

D. nihonkaiensis cytochrome c oxidase subunit 1 (*cox1*) genes DNA was quantified by one-step qPCR using the primers previously described (Sequence (5′→3′) forward CTTTGTTGTCTGGCCTTCCT, and reverse ATGATAAGGGAYAGGRGCYCA) (*2*). The specificity of these primer/probe sets had been confirmed by others (*2*). Samples were analyzed using the Bio-Rad iTaq Universal SYBR Green One-Step Kit (Bio-Rad, USA) in 20-μL reactions run at 50°C for 10 min and 95°C for 1 min, followed by 40 cycles of 95°C for 10 s and 60°C for 30 s per the manufacturer's recommendations. *D. nihonkaiensis* DNA concentrations were determined using a standard curve as previously described and presented as parasite DNA copies. For the generation of the standard curve, six 10-fold serially diluted positive control DNAs of *cox1* gene (concentration range, 5.0 $\times 10^5$–5.0 $\times 10^0$ copies/2.5 μL) were included in each qPCR run to obtain a standard curve. The primer set generated a standard curve with an efficiency of 97.8% and R^2 of 0.971. Threshold cycle (Ct) values above 40 were considered negative for *cox1* gene.

2.5. Sequence and phylogenetic analyses

Sequences of a partial fragment of the *cox1* gene were used for genetic analysis because of the numerous available sequences from the definitive and fish intermediate host species, and because of its usefulness for genetic differentiation of *D. nihonkaiensis* (*2*). A portion of the *cox1* gene (approximately 710 bp) was amplified using the primer set: forward (5′-TTG ATC GTAAAT TTG GTT C-3′); reverse (5′ -AAA GAA CCT ATT GAA CAA AG-3′) (*2*). PCR amplification was performed in a volume of 25 μL using TaKaRa EX Taq Hot Start Version containing 10 PCR buffer, 20 mM $MgCl_2$, 2.5 mM of each dNTP, 5 units/μL of Takara Ex Taq HS DNA polymerase (TaKaRa Shuzo Co. Ltd., Shiga, Japan), 0.5 μM of each primer, and 2.5 μL of DNA sample. After denaturation at 94°C for 5 min, amplification was carried out by 35 cycles consisting of denaturation at 94°C for 30 s, annealing at 52°C for 30 s, and elongation at 72°C for 1 min, followed by a final extension step at 72°C for 7 min. Reactions were performed in a thermocycler (GeneAmp PCR System 9700 or 2720; Applied Biosystems, USA). Aliquots of the PCR products were separated by electrophoresis on a 3% agarose gel and were visualized under UV light after staining with ethidium bromide. The PCR products were purified using either the QIAquick Gel Extraction Kit or the QIAquick PCR Puri cation Kit (Qiagen Inc., Germany), and DNA sequencing was performed on an automated sequencer (ABI3130; Applied Biosystems, USA) using a BigDye Terminator v3.1 Cycle Sequencing kit with the primer sets used in the PCR. Sequence chromatograms from each strand were inspected using Sequencher DNA Sequence Analysis Software Version 4.1 (Gene Codes Corp., USA). Sequence alignment was done using MEGA-11 Software. Best DNA models for maximum likelihood estimation were identified and phylogenetic trees were constructed (Hasegawa-Kishino-Yano Model with uniform rates among sites) with 1000 bootstrap replications (*4,5*). A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.1000)).

2.6. Data visualization

Data were analyzed and plotted using the ggplot2 3.3.5, packages of R program version 4.1.0 (*6*).

3. Results and Discussion

Between 2022 and 2023, four cases of *D. nihonkaiensis* infection were identified, along with one suspected case, affecting individuals aged 13 to 54 (Table 1). All patients reported intermittent gastrointestinal symptoms such as stomach pain, constipation, gas, nausea, or loose stools (Table 1). The infections were linked to the consumption of raw or undercooked wild fish (Cherry salmon, *Oncorhynchus masou*) in three cases, with one case also involving undercooked bear meat. The patients who consumed undercooked bear meat had no history of consuming raw or undercooked cherry salmon (*O. masou*) in the past five years. The patient, an avid wildlife hunter, reported occasionally cleaning bear carcasses in the wild after hunts. It is therefore plausible that exposure during the handling of bear meat and gastrointestinal contents contributed to contamination with tapeworm eggs. Microscopic examination confirmed the presence of tapeworm eggs (ova) in three cases, and PCR testing identified *D. nihonkaiensis* in all four. The suspected case had a history of sharing food with a domesticated cat (the cat has a previous history of Tapeworm infection, according to the patient). However, the suspected case refused to provide a faecal sample and declined any treatment. None of the patients had any underlying diseases or medical conditions.

Treatment with praziquantel, administered at doses of 10–20 mg/kg as a single dose, was effective in all treated cases. Following treatment, three patients excreted tapeworms measuring between 76 cm and 210 cm in length, with a total of eight worms excreted. The fourth patient did not provide any excreted worms for examination. Follow-up at three months showed no complications and negative faecal tests for eggs in all patients.

Environmental surveillance revealed that *D. nihonkaiensis* positive samples were spread across the region, with bear feces and Cherry salmon (*O. masou*) fish samples testing positive for *D. nihonkaiensis* in multiple areas of the prefecture (Figure 1a). Overall, 33.3% of bear faecal samples and 21.8% of wild fish meat samples (Cherry salmon, *O. masou*) tested positive

Table 1. Demographics and clinical details of *D. nihonkaiensis* cases between Winter 2022 and Spring 2023

Human sample	Age	Sex	Microscopic identification for eggs	Tapeworm/ proglottids in feces	PCR identification	Initial symptoms	Exposure history	Treatment	3-month follow-up
H3902980	13	Male	Postive	8	*D. nihonkaiensis*	Intermittent stomach ache or stomach pain, upset stomach, nausea, weight loss.	Eating raw fish (Cherry salmon, *Oncorhynchus masou*) one year ago	Praziquantel 20mg/kg single dose	Fecal negative for eggs/no complication
H3902981	45	Male	Positive	2	*D. nihonkaiensis*	Intermittent stomach ache or stomach pain, gas.	Eating cooked bear meat, and raw fish (Cherry salmon, *Oncorhynchus masou*) six months ago	Praziquantel 10mg/kg single dose	Fecal negative for eggs/no complication
H3902982	54	Male	Postive	2	*D. nihonkaiensis*	Intermittent stomach ache or stomach pain, gas.	Eating raw fish(Cherry salmon, *Oncorhynchus masou*) regularly	Praziquantel 10mg/kg single dose	Fecal negative for eggs/no complication
H3902983	53	Female	-	-	*D. nihonkaiensis*	Loose stools	Eating raw fish (Cherry salmon, *Oncorhynchus masou*) regularly	Praziquantel 10mg/kg single dose	Fecal negative for eggs/no complication

for *D. nihonkaiensis* (Figure 1b). The phylogenetic analysis of *D. nihonkaiensis* samples from human, bear feces, and fish revealed close genetic relationships, indicating a shared transmission cycle among these hosts (Figure 1c). Samples from bears, fish, and humans clustered into distinct but closely related groups, suggesting minimal genetic divergence within the local tapeworm population.

The observed increase in *D. nihonkaiensis* cases between winter 2022 and spring 2023, coupled with the environmental samples (Cherry salmon, *O. masou*) testing positive during this period, suggests a potential rise in the risk of human infection. This temporal correlation highlights a significant aspect of epidemiology, where environmental conditions and biological activity may influence infection rates (*1,2*). The heightened environmental presence of *D. nihonkaiensis* in bear and wild Cherry salmon (*O. masou*) samples during these months could indicate a seasonal factor, which would mean climate change will contribute to increased human exposure. Given the seasonal migration patterns of cherry salmon in spring and the availability of immature chum salmon during summer months in northern Japan, these periods may represent peak risk windows for *D. nihonkaiensis* transmission.

The genetic analysis revealing close relationships between *D. nihonkaiensis* strains from human, wild fish (Cherry salmon, *O. masou*), and bear sources supports the hypothesis of a shared environmental source of infection (*7*). This genetic proximity indicates that contamination in the food chain, particularly through raw or undercooked wild fish, is a critical pathway for transmission to humans (*1,2,7*). The linkage between bear and wild fish Cherry salmon (*O. masou*) reservoirs with human cases underscores the need for targeted public health interventions focusing on these key reservoirs (*8*). Based on our findings, we also hypothesize that the natural host of *D. nihonkaiensis* maybe bear (and potentially other terrestrial animals), with wild fish Cherry salmon (*O. masou*) becoming contaminated through exposure to feces from infected hosts. In forested freshwater ecosystems, bears frequently defecate near riverbanks, shedding eggs or proglottids of zoonotic parasites such as *Dibothriocephalus* spp., the causative agents of diphyllobothriasis (fish tapeworm infection). These feces contaminate the surrounding environment and can attract coprophagous and saprophagous flies. Flies, acting as mechanical vectors, may carry parasite eggs to aquatic habitats or directly contaminate the insect populations residing in or near the river, including chironomids, mayflies, and stoneflies. Juvenile cherry salmon (*O. masou*), which remain in upstream freshwater zones for one to three years before their seaward migration, feed heavily on these aquatic and airborne insects (*9,10*). This feeding behavior creates a route for the salmon to become intermediate or paratenic hosts of

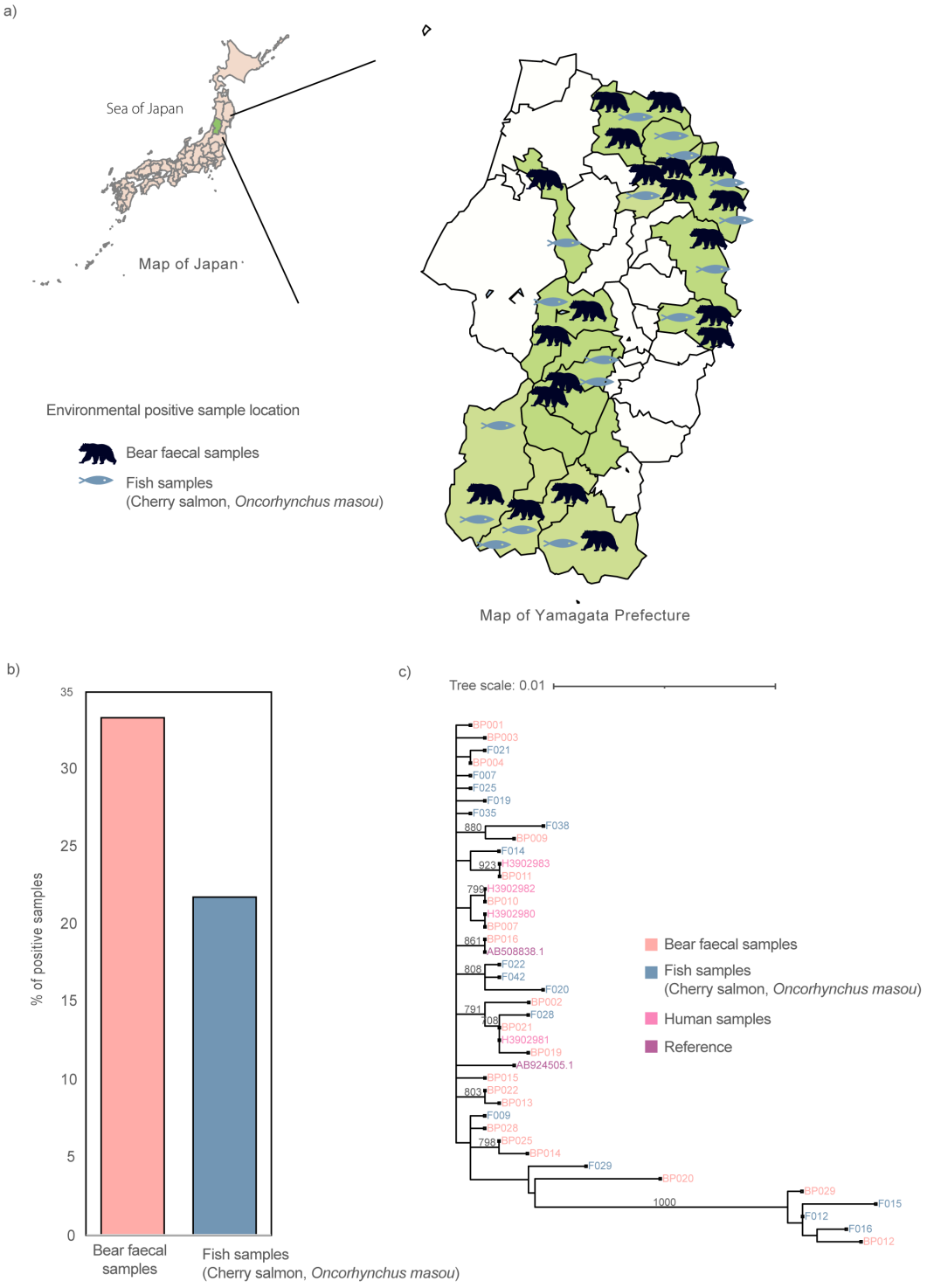

Figure 1. a) Number of *D. nihonkaiensis* clinical cases between Winter 2022 and Spring 2023, b) Locations in Yamagata Prefecture, with bear faecal and fish samples testing positive for *D. nihonkaiensis*, c) Percentage of bear faecal and wild fish meat samples tested positive for *D. nihonkaiensis*, d) The phylogenetic analysis of *D. nihonkaiensis* samples from human, bear feces, and fish (Cherry salmon, *O. masou*).

zoonotic tapeworm larvae, especially in areas where the parasite life cycle includes copepods or insect larvae as intermediate hosts (*10*). Transmission among bears may occur through their dietary habits, which include the consumption of plants and fish that have been exposed to parasitic contamination *via* faecal matter.

Additionally, studies and data from the Japan Meteorological Agency confirm a consistent rise in both sea surface and river water temperatures around Japan, including in Yamagata Prefecture, over the past decade (*11*). Notably, the shortening of winter seasons, likely driven by rising regional temperatures, has significant implications for the behavior of black bears. Warmer and shorter winters delay the onset of hibernation and shorten

hibernation duration, resulting in increased roaming activity by bears in search of food. This behavioral shift extends the window during which bears defecate in the environment, depositing tapeworm eggs near rivers and streams and sustaining environmental contamination for longer periods.

Yamagata is historically known for its association with the Matagi, a traditional group of indigenous mountain hunters skilled in sustainable bear hunting (*12*). Bear meat remains a culturally valued food in the region, and it is often consumed in rural communities. Moreover, in Japan, people usually do not consume raw freshwater fish, following ancient wisdom that such fish are likely contaminated with parasites, including tapeworms. However, Cherry salmon (*O. masou*) are exceptions and are eaten raw. This aligns with the human infections in this study, as all three cases involved individuals who consumed raw or undercooked Cherry salmon (*O. masou)* which is abundant in Yamagata's rivers (*13*).

Given the correlation between human cases and the seasonal increase in positive environmental samples, public health efforts should prioritize education on the risks associated with consuming raw or undercooked fish (*7,8*). This is especially pertinent in regions where *D. nihonkaiensis* is known to be prevalent (*1,2,14,15*). Increasing awareness about safe food handling and preparation practices could help mitigate the risk of infection (*1,2,14*). Interestingly, in Japan, praziquantel is not recommended for tapeworm infections in medical guidelines and insurance reimbursements, but worldwide, it is a first-line drug for cestodes (*14-16*). However, the Parasitology Society of Japan recommended the use of praziquantel in its recommendations. Praziquantel is considered to be a clinically effective treatment for cestodoses, has a low burden on patients, and can be safely administered on an outpatient basis (*15,16*). This discrepancy in its use between Japan and the global medical community raises questions about local clinical practices and treatment guidelines (*17*). The variation could stem from differences in parasite strains, treatment preferences, or regulatory policies. Nevertheless, its broad efficacy, low side effects, and ease of administration have made it a staple in the management of parasitic infections in most countries (*14-16*). Further research may help clarify whether alternative treatments are more appropriate in specific regions or whether praziquantel's widespread acceptance should extend to areas where it is not currently recommended.

Importantly, our study does not imply that all bears, fish, or river water are inherently contaminated or dangerous; rather, it highlights that parasites like *D. nihonkaiensis* are naturally present in the environment. The recent increase in infections likely reflects the combined influence of changing climate conditions and human behaviors such as consuming raw wild meat or fish on the dynamics of parasite transmission. With appropriate hygiene practices, food safety awareness, and ecological understanding, such zoonotic risks can be effectively mitigated without vilifying wildlife or natural ecosystems. We acknowledge, however, that our findings are based on a small number of confirmed cases and suspected case, all restricted to Yamagata Prefecture. This limited sample size may introduce randomness in the interpretation of clinical symptoms and infection routes, and the results should therefore be interpreted with caution when extrapolating to other regions of Japan where dietary habits and ecological contexts may differ. Additionally, while our seasonal risk hypothesis is supported by the clustering of cases between winter 2022 and spring 2023, we did not have access to long-term environmental surveillance data (*e.g.*, parasite detection from 2017–2021 or host distribution records from earlier years). Thus, we cannot yet determine whether this increase reflects a long-term trend in transmission dynamics or an isolated event.

In summary, the results underscore the importance of integrating environmental surveillance data with human infection trends to better understand and manage the risk of parasitic diseases. Public health strategies should be adapted to address seasonal, environmental, and climate change factors, focusing on preferred treatment in Japan alongside reducing exposure to contaminated food sources and enhancing community education about the risks.

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Ethical approval: This study was conducted in

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